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Evaluation of a novel proximity ligation assay for the sensitive and rapid detection of foot-and-mouth disease virus

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Abstract

A novel proximity ligation assay (PLA) using a pan-serotype reactive monoclonal antibody was developed and evaluated for the detection of foot-and-mouth disease virus (FMDV) in clinical samples collected from field cases of disease. The FMDV-specific PLA was found to be 100 times more sensitive for virus detection than the commonly used antigen capture-ELISA (AgELISA). As few as five $TCID_{50}$ were detected in individual assays, which was comparable with the analytical sensitivity of real-time RT-PCR. Although this assay was capable of detecting diverse isolates from all seven FMDV serotypes, the diagnostic sensitivity of the PLA assay was lower than real-time RT-PCR mainly due to a failure to detect some SAT 1, SAT 2 and SAT 3 FMDV strains. In conclusion, this new PLA format has high analytical sensitivity for the detection of FMDV in clinical samples and may prove valuable as a rapid and simple tool for use in FMD diagnosis.

Keywords: Proximity ligation assay (PLA); Foot-and-mouth disease (FMD); Diagnostic test; Pathogen detection

1. Introduction

Foot-and-mouth disease virus (FMDV) is a member of the family Picornaviridae and exists as seven immunologically distinct serotypes (A, O, C, SAT 1, SAT 2, SAT 3 and Asia 1). The disease caused by this virus is endemic in many regions of Africa, Asia and South America, often causing extensive epidemics in domesticated cloven-hoofed livestock. In

Abbreviations: PLA, proximity ligation assay; mAb, monoclonal antibody; S/N, signal to noise; $TCID_{50}$, tissue culture infectious dose; PBS, phosphate-buffered saline; RT-PCR, reverse transcriptase PCR; Ct, threshold cycle.

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addition, more than 70 species of wild mammals belonging to more than 20 families are susceptible to infection. The virus can also cause persistent infection of the pharynx in cattle, sheep, goats, and the other ruminants which can complicate the epidemiology and control of the disease. The highly contagious nature of FMD motivates great urgency in laboratory diagnostic analysis, especially when the virus is introduced into countries previously classified as FMD-free. Such was the case in the United Kingdom in 2001 when the FMD epidemic resulted in devastating losses to the food, farming, tourism and leisure industries. (Thompson et al., 2002).

Diagnosis of FMD depends upon early recognition of the clinical signs of disease in the field, followed by confirmation of the presence and serotype-specificity of FMDV in the laboratory. Established laboratory assays for the detection of FMDV include virus isolation (VI) in cell culture (Snowdon, 1966), antigen capture-ELISA (Ferris and Dawson, 1988), and reverse transcription polymerase chain reaction (RT-PCR; Reid et al., 2002, 2003). VI has high sensitivity and is considered the gold standard method but it can be slow and laborious. In contrast, antigen ELISA is more rapid but has lower sensitivity and therefore cannot be reliably used to confirm negative cases. RT-PCR has recently been shown to be a rapid method with still higher diagnostic sensitivity than VI (Shaw et al., 2004; King et al., 2006). Furthermore, since FMD cannot be differentiated clinically from the other vesicular viral diseases of swine, e.g., swine vesicular disease (SVD), vesicular stomatitis (VS) and vesicular exanthema of swine (VES), differential diagnosis is an important aspect of laboratory investigation.

The aim of this study was to evaluate the proximity ligation assay (PLA) for detection of FMDV in clinical samples. This is a new technique that has been used for detection of proteins and microorganisms in complex biological samples and has already been shown to be as sensitive as more established nucleic acid detection assays such as PCR (Gustafsdottir et al., 2006). The basis of the PLA is that FMDV specific-antibodies binding target proteins are coupled to oligonucleotide strands. These oligonucleotides can be joined by ligation when two or more such reagents are brought into proximity by binding to the same target molecule or target molecule complex (Fig. 1). The DNA ligation products are subsequently detected by PCR amplification using fluorogenic probes to detect the amplified product.

2. Material and methods

2.1. Propagation of FMDV cell culture-grown antigen and determination of $TCID_{50}$

$TCID_{50}$ values for cell culture-grown viruses were calculated according to the method described by Kärber (1979).

2.2. Monoclonal antibody (mAb) 1F10 for use as a binding ligand in the PLA

The mAb 1F10 was chosen for this study due to its capability to recognise all seven FMDV serotypes in both a trapping ELISA (Samuel et al., 1991) and a sandwich ELISA (Brocchi et al., 1993). In principle, in the trapping ELISA, the mAb 1F10 reacted with each of the seven serotypes preliminarily immune-captured onto the solid phase by an homologous rabbit antiserum; in the sandwich ELISA, the mAb 1F10 was used either as antigen-capture antibody or as the second antibody conjugated with peroxidase.

The mAb 1F10 was obtained from a mouse immunised with the FMD virus type O, strain UK31/2001. The mAb belongs to IgG1 isotype, does not neutralise virus infectivity and is presumably directed against a conformation-dependent epitope since it does not recognise isolated viral proteins in immuno-blotting test (data not shown).

2.3. Biotinylation of mAb and preparation of proximity ligation probes

Biotinylation was performed according to the manufacturer's instructions (Roche Diagnostics Corp, Germany). Briefly, D-biotin-*N*-hydroxysuccinimide ester was mixed with the antibody in a 10-fold molar excess and with a volume ratio of 1:10. The solution was incubated for 4 h at room temperature with continuous agitation. The biotinylated mAbs were then dialysed thoroughly in phosphate buffered saline (PBS, pH 7.4) to remove unbound biotin. Proximity ligation probes were constructed by combining the free 3′ or 5′ ends of strepatividin–